whether such bubbles actually facilitate the formation of microcircles.

Although transient bubbles or related structures might increase the probability of forming small circles, longer DNA molecules are probably similarly dynamic. Increased flexibility, however, is not the universal panacea for either DNA wrapping or circularization. It comes with a cost. Static bends which allow a DNA molecule to easily adopt an appropriate configuration can considerably enhance both circularisation and nucleosome formation [18]. Nevertheless when the flexibility of a DNA sequence is increased at the expense of loss of static bending, DNA wrapping is impaired rather than enhanced [14].

This is presumably because the more flexible a sequence is, the more configurations it can easily adopt, so that the entropic penalty on wrapping is consequently greater. This implies that the occurrence of transient, hyperflexible lesions might be detrimental to the formation of larger circles, where the average bend angle per double helical turn is within the normal fluctuations for maintaining the stacking consistent with smooth bending. As the calculation of the torsional flexibility from experimentally determined cyclization rates could include a component, either positive or negative, from the hyperflexible lesions the relative contributions of discontinuous and smooth fluctuations clearly need to be refined.

To what extent are these considerations applicable to DNA in chromatin — whether eukaryotic or bacterial? Much of the genomic DNA is packaged, and so constrained, by non-specific DNA binding proteins. Yet even when not complexed with proteins, other stabilising factors — divalent cations and/or polyamines — could limit the dynamic flexibility of DNA. Moreover eukaryotes and bacteria have both evolved functionally analogous but structurally distinct proteins — HMGB proteins in eukaryotes and HU in bacteria — that facilitate circularisation in part by introducing one or more kinks into the DNA. However, HMGB-induced circularisation of DNA, even for very small circles, appears to a first approximation to be independent of helical phase, implying that these proteins not only bend the DNA but also increase torsional flexibility [19,20]. One possible implication is that cells adopt an authoritarian attitude towards their most precious asset and act to suppress dynamic random melting events that could promote deleterious events such as adventitious transcription initiation.

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# Chromosome Segregation: Aurora B Gets Tousled

**Aurora B kinases play important roles during mitosis in eukaryotic cells; new work in *Caenorhabditis elegans* has identified the Tousled kinase TLK-1 as a substrate activator of the model nematode's Aurora B kinase AIR-2 which acts to ensure proper chromosome segregation during cell division.**

**Christopher T. Richie and Andy Golden**

For more than three decades, phosphorylation of histone H3 has been used as a marker for entry of a eukaryotic cell into meiosis or mitosis: it correlates with the condensation of chromosomes that is a prerequisite for their movement and thus proper segregation. Over the years, numerous kinases have been implicated in histone

H3 phosphorylation. Recently, attention has focused on two kinase families as the responsible enzymes: the Aurora/Ipl1 family identified in yeast and *Caenorhabditis elegans* [1]; and the Tousled kinase in human cells [2].

Tousled kinase was shown to phosphorylate histone H3 during mitosis in human cells [2]. In *C. elegans*, however, the Tousled-like kinase, TLK-1, does not appear to have that ability [3,4]: in this species, the job of histone H3 kinase appears to be taken by an Aurora B ortholog, AIR-2, which has multiple roles in cell division in the nematode [1]. The mechanisms of action of these kinases and their regulators are largely unknown.

As well as phosphorylating histone H3, in many organisms Aurora kinases phosphorylate substrates that then serve as their own positive regulatory subunits [5] — so-called 'substrate activators'. Until recently, the only known substrate activator of an Aurora B kinase was the chromosomal passenger protein INCENP/ICP-1 [6,7]. But as reported in this issue of *Current Biology*, Han *et al*. [3] have now shown that TLK-1 is a novel substrate activator of AIR-2, enhancing the latter's ability to phosphorylate histone H3. This finding suggests that the Tousled kinases may act in concert with Aurora B orthologs in other organisms to affect histone H3 phosphorylation during cell cycle progression.

*C. elegans* has two Aurora/Ipl1-related kinases: AIR-1/Aurora A and AIR-2/Aurora B. AIR-1 associates with mitotic centrosomes and its depletion by RNA interference (RNAi) results in aberrant spindles and chromosome segregation defects [7]. AIR-2 has the defining property of a chromosomal passenger protein of moving from the metaphase kinetochores to the anaphase central spindle and midbody. Loss of AIR-2 function through RNAi depletion or *air-2* mutation results in multiple defects, affecting extrusion of the 'polar body' products of meiosis, chromosome segregation during meiosis and mitosis, and cytokinesis [8,9]. AIR-2 is often found physically associated with BIR-1/Survivin and ICP-1 [5] in what is known as the ABI complex [10]. A fourth subunit of this complex, CSC-1/borealin, was recently identified in *C. elegans* and vertebrate cells [5].

*C. elegans* TLK-1 is a multi-functional kinase that acts at several stages of the cell cycle; it also has an essential role in early embryonic transcription [4]. TLK-1-depleted embryos arrest around the 100-cell stage — about the same time as embryos deficient in RNA polymerase II [11]. TLK-1 affects histone and RNA polymerase II modifications that are correlated with transcriptional elongation [4]. Tousled kinases from *C. elegans, Drosophila* and humans also phosphorylate Asf1 [3,12,13], a protein involved in chromatin assembly during DNA replication [14,15]. Furthermore, Tousled is inhibited in response to a DNA damage checkpoint [16,17].

In *Drosophila*, Tousled-deficient *tlk* mutant embryos display anaphase bridges during chromosome segregation and reduced histone H3 phosphorylation [12], suggesting that Tousled also plays roles later in the cell cycle. These later phenotypes are reminiscent of Aurora B-deficient phenotypes [7]. The new findings of Han *et al*. [3] clearly establish a functional connection between Tousled and Aurora B for the first time, and demonstrate that these two proteins function together to influence chromosome segregation.

Using a yeast two-hybrid assay, Han *et al*. [3] initially identified TLK-1 as an AIR-2 binding factor. As AIR-2 and TLK-1 are both kinases, it was necessary to determine whether one of the kinases was a substrate of the other. Both kinases were active when expressed as recombinant proteins in bacteria. The authors found that TLK-1 does not phosphorylate AIR-2, but AIR-2 does phosphorylate TLK-1 in the presence of ICP-1. The authors mapped the AIR-2 phosphorylation site on TLK-1, identifying serine 634 (S634) as the major site.

*In vitro* kinase assays showed that phosphorylation of TLK-1 by AIR-2/ICP-1 stimulated the former's kinase activity, and that this stimulation was abrogated by a serine-to-alanine substitution at residue 634 (S634A). TLK-1's kinase activity was enhanced over wild-type when S634 was substituted with glutamic acid (S634E), which serves as a phospho-mimetic variant. The kinase activity of this S634E variant was independent of the influence of AIR-2/ICP-1.

The *in vitro* kinase assays with TLK-1 consistently revealed an increase in AIR-2 autophosphorylation and ICP-1 phosphorylation, suggesting that association with TLK-1 stimulates the AIR-2 kinase. To test whether TLK-1 is a substrate activator of AIR-2, Han *et al.* [3] added wild-type TLK-1 to AIR-2 and ICP-1, and found that it enhances AIR-2/ICP-1-mediated histone H3 phosphorylation. The S634A mutation of TLK-1 prevented this enhancement, suggesting that only phospho-TLK-1 can function as an activator. In support of this interpretation, the S634E mutant of TLK-1 showed the best enhancement of AIR-2 kinase activity towards histone H3. Surprisingly, a kinase-dead version of TLK-1 also acted as a substrate activator. Thus, TLK-1 is a substrate activator of AIR-2 and can function in this capacity independent of its own kinase activity.

So where in the embryo is phospho-TLK-1 localized? Previous studies by the same group [4] showed that TLK-1 localizes to interphase and prophase nuclei. By employing antibodies specific to the phosphorylated (S634) epitope of TLK-1, the authors established that the phosphorylated species is restricted to a confined volume — 'halo' — closely associated with mitotic chromatin during prometaphase and metaphase in early embryos. AIR-2 itself is localized to chromosomes during these stages [8] at a time when AIR-2 phosphorylates histone H3 [1]. The phospho-TLK-1

localization pattern is dependent on AIR-2 and ICP-1 expression *in vivo.*

Interestingly, TLK-1's role as a substrate activator seems to depend on the presence of ICP-1, another substrate activator of AIR-2. AIR-2 thus assembles into a complex with two substrate activators for enhanced histone H3 kinase activity. The ability of AIR-2 to phosphorylate TLK-1, and the ability of TLK-1 to activate AIR-2, are both dependent on the presence of ICP-1. What other proteins associate with this complex remains to be explored. Given that AIR-2 and ICP-1 are often found associated with BIR-1 and CSC-1 [5], it will be interesting to determine whether these proteins are also present in AIR-2/TLK-1 complexes (though BIR-1 and CSC-1 do not appear to be substrate activators themselves). As TLK-1 does not have a localization pattern typical of these other chromosomal passenger proteins, it seems unlikely that TLK-1 is a subunit of this complex at all times.

To see whether TLK-1 functions in processes regulated by AIR-2, Han *et al*. [3] looked for, and found, a genetic interaction *in vivo*. They used RNAi to knock down TLK-1 expression in a strain carrying a temperature-sensitive hypomorphic allele of *air-2*. At the permissive temperature, this mutant displays a low level of abnormal anaphase events in young embryos [9]. Although *tlk-1* RNAi alone has no effect on chromosome segregation in wild-type early embryos, in the *air-2* mutant, at the permissive temperature, it more than doubled the incidence of abnormal anaphase events. Embryos with reduced levels of both TLK-1 and AIR-2 thus showed enhanced mitotic defects, further suggesting that these two kinases function together during early embryonic cell divisions.

TLK-1-depleted *air-2* mutant embryos also showed dramatic reductions in phospho-histone H3 levels. In wild-type or TLK-1-depleted early embryos, AIR-2 kinase activity is presumably above some threshold to permit normal mitosis. But compromised AIR-2 activity, as in the temperature-sensitive mutant, shows a dependence on TLK-1, such that TLK-1 depletion enhances the mitotic defects of *air-2* mutants. This enhancement is indicative of an *in vivo* interaction.

What is the likely function of TLK-1 as a substrate activator of AIR-2? Is it a necessary component of an AIR-2 complex required for histone H3 phosphoryation? TLK-1 depletion does not appear to affect phospho-histone H3 levels in early *C. elegans* embryos [3,4], yet in *Drosophila* [12] and cultured mammalian cells [18], reductions in Tousled activity correlate with decreases in phospho-histone H3 levels. This decrease could be due to a decrease in the number of M-phase nuclei [12], but equally it might reflect Tousled action as a substrate activator of Aurora B that stimulates histone H3 phosphorylation.

To address whether the histone H3 phosphorylation activity attributed to Tousled in other organisms occurs in association with Aurora B, it will be necessary to re-examine possible effects on Aurora B activity when Tousled activity is reduced. But why are phospho-histone H3 levels not reduced in *C. elegans* TLK-1-depleted embryos? Either TLK-1 is not essential for wild-type AIR-2 to phosphorylate histone H3 or *tlk-1* RNAi does not effectively deplete all of the TLK-1 in the embryo. The true requirement of TLK-1 for histone H3 phosphorylation in *C. elegans* is likely to come from the analysis of a conditional or null allele of *tlk-1*.

Aurora kinases associate with different substrate activators to carry out their different roles during the cell cycle. Aurora A associates with Ajuba for mitotic entry [19] and with TPX-2 for spindle assembly [7]. Aurora B associates with chromosomal passenger proteins during metaphase and anaphase. *C. elegans* TLK-1 is the latest to join the list of proteins that associate with, and activate, a member of the Aurora kinase family. When Tousled activity is impaired in *Drosophila, C. elegans* and mammalian cells, chromosome segregation defects and changes in histone H3 phosphorylation are apparent [1,3,12,18]. Whether Aurora B orthologs associate with Tousled in other organisms remains to be determined, as does the precise subcellular localization of the AIR-2/ICP-1/phospho-TLK-1 complex in *C. elegans* early embryos. Though histone H3 phosphorylation remains a convenient marker of AIR-2/TLK-1 activity, the identification of other physiologically significant substrates of AIR-2 remains an important goal. This knowledge will aid in our understanding of how the various localizations and compositions of Aurora B complexes influence substrate selection and chromosome behavior.

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# Immunology: How Do T cells Recognize Antigen?

**T cells recognize small fragments of microorganisms (antigens) on the surface of other cells using T cell antigen receptors. The mechanism by which these receptors signal into T cells is controversial, but two recent studies provide important new clues.**

**Kaushik Choudhuri, Alice Kearney, Talitha R. Bakker and P. Anton van der Merwe**

The recognition of microorganisms by T cells is the central event in the adaptive immune response to infection. Each T cell expresses a unique T-cell antigen receptor (TCR), which recognizes microorganism-derived peptides presented on cell-surface major histocompatibility complex (MHC) molecules. The binding of TCRs to peptide-bound MHC (pMHC) with high affinity leads to a series of signalling events culminating in cellular responses such as proliferation, differentiation and secretion of cytokines and growth factors. Although the TCR signal transduction pathways have been intensively studied, it is still not clear how binding of pMHC to TCR is initially communicated across the T-cell plasma membrane, a process termed TCR triggering [1–3]. We review here two recent studies that shed light on the TCR triggering mechanism and help to reconcile much of the existing conflicting data [4,5].

TCRs are composed of two transmembrane subunits ($\alpha$ and $\beta$) with very short cytoplasmic tails that are non-covalently associated with several signalling subunits. The latter have cytoplasmic motifs that are modified by tyrosine phosphorylation and recruit cytoplasmic signalling molecules. A typical TCR$\alpha\beta$ is associated with at least six signalling subunits, including CD3$\varepsilon\gamma$ and CD3$\varepsilon\delta$ heterodimers and a TCR$\zeta\zeta$ homodimer. Also important for signalling are the CD8 or CD4 'coreceptors', which bind to the non-variable MHC portion of the pMHC class I (pMHCI) and pMHC class II (pMHCII) complexes, respectively, and whose cytoplasmic domains bind to the tyrosine kinase Lck.

Models proposed for TCR triggering can be classified into three categories according to whether they invoke aggregation, conformational change, or segregation of the TCR–CD3 complex as the primary mechanism of signal transduction (Figure 1). Aggregation models are supported by the observation that artificial aggregation of TCR–CD3, using either antibodies or multivalent forms of soluble agonist (high affinity) pMHC, is sufficient for TCR triggering. However, the paucity of agonist pMHC on cells and the more recent finding that a single agonist pMHC is alone sufficient for TCR triggering in $CD4^+$ or $CD8^+$ T cells [6,7] seemed to rule out aggregation mechanisms.

Now Krogsgaard *et al.* [4] report that MHC class II molecules bearing peptides derived from endogenous proteins (endogenous pMHCII), which are presumably low affinity and unable to induce TCR triggering on their own, enhance TCR triggering by MHC class II molecules bearing agonist peptides. This was demonstrated using soluble agonist–endogenous pMHCII heterodimers as well as mixtures of agonist and endogenous pMHC on artificial membranes and cells. Because endogenous pMHCII is highly abundant on antigen-presenting cells, it is indeed plausible that TCR aggregation could be induced by such a mixture of endogenous and agonist pMHC.

Krogsgaard *et al*. [4] go on to show, using mutated forms of pMHCII, that CD4 binding to agonist pMHCII was required for this effect whereas CD4 did not